

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph no. 43 with the following amended paragraph:

Figure 8A shows the cDNA sequence and the deduced amino acid sequence (SEQ ID NO:95) of the light chain variable region for the murine My9-6 antibody. The three CDRs are underlined.

Please replace paragraph no. 44 with the following amended paragraph:

Figure 8B shows the cDNA sequence and the deduced amino acid sequence (SEQ ID NO:96) of the heavy chain variable region for the murine My9-6 antibody. The three CDRs are underlined

Please replace paragraph no. 171 with the following amended paragraph:

The solvent accessibility of the variable region residues for a set of antibodies with solved structures was used to predict the surface residues for the murine My9-6 antibody variable region. The amino acid solvent accessibility for a set of 127 unique antibody structure files (Figure 3) were calculated with the MC software package (Pedersen et al., 1994, *J. Mol. Biol.* 235(3):959-973). The ten most similar light chain and heavy chain amino acid sequences from this set of 127 structures was determined using sequence alignment software on the NCBI website (<http://www.ncbi.nlm.nih.gov/BLAST/>). The average solvent accessibility for each variable region residue of these ten antibody variable regions was calculated with an Excel spreadsheet and positions with greater than a 30% average accessibility were considered surface residues. Positions with average accessibilities of between 25% and 35% were further considered by calculating the individual residue accessibility for only those structures with two identical residues flanking on either side.

Please replace paragraph no. 173 with the following amended paragraph:

A molecular model of the variable region of murine My9-6 was generated using the Oxford Molecular software package AbM. The antibody framework was built from structure files for the antibodies with the most similar amino acid sequences (1sbs for the light chain and 1bbj for the heavy chain) and the non-canonical CDRs were built by searching a C- α structure database containing non-redundant solved structures. Models were viewed with the

GlaxoSmithKline Swiss-Pdb Viewer and residues that lie within 5 \AA of a CDR were determined.

Please replace paragraph no. 175 with the following amended paragraph:

The surface positions of the murine My9-6 variable region were compared to the corresponding positions in human antibody sequences in the Kabat database (Johnson and Wu, 2001, Nucleic Acids Res. 29(1):205-6). The antibody database management software SR (Searle, 1998) was used to extract and align the surface residues from natural heavy and light chain human antibody pairs. The human antibody variable region surface with the most identical surface residues, with special consideration given to positions that come within 5 \AA of a CDR, was chosen to replace the murine My9-6 antibody variable region surface residues.

Please replace paragraph no. 178 with the following amended paragraph:

Humanization primer sets were designed to make the 6 amino acid changes required for all versions of huMy9-6 and additional primers were designed to alternatively change the four 5 \AA residues (Figure 4). PCR reactions were performed on an MJ Research thermocycler with the following program: 1) 94°C, 1 min; 2) 94°C, 15 sec; 3) 55°C, 1 min; 4) 72°C, 1 min; 5) cycle back to step #2 29 times; 6) finish with a final extension step at 72°C for 4 min. The PCR products were digested with their corresponding restriction enzymes and cloned into the pBluescript cloning vectors. Clones were sequenced to confirm the amino acid changes.

Please replace paragraph no. 194 with the following amended paragraph:

HL-60 membranes, prepared as described above, were dried at a starting concentration of 10 $\mu\text{g}/\text{mL}$ in deionized water onto the polystyrene surface of Immulon 2 96-well assay plates (Dynex Laboratories, Chantilly, VA), using a Labconco vacuum desiccator (Labconco, Corp, Kansas City, MO), overnight at ambient temperature. The ELISA plate was then blocked with a 1% fraction V bovine serum albumin (Sigma-Aldrich, Inc., St. Louis, MO, cat. no. A-3294), 0.05% Tween-20 (Sigma-Aldrich, Inc., St. Louis, MO, cat. no. P-2287) solution in Tris-buffered saline (50 mM Tris-HCl, 150 mM sodium chloride, pH 7.5, TBS), 300 μL per well, at 37°C for 1 hour. Following this blocking step, the plate was drained of blocking buffer and blotted onto paper towels. Two, threefold serial dilutions of test or control reagents were prepared in blocking buffer, in quadruplicate, starting at $3.13 \times 10^{-9} \text{ M}$ titrated down to $5.29 \times 10^{-14} \text{ M}$ on a

flexible 96-well assay plate. The negative control wells contained blocking buffer alone. 50 µL of each dilution was transferred to designated wells on the membrane-coated assay plate that was then incubated overnight at 4°C. The well contents were then aspirated into a waste flask containing 10% (v/v) bleach, and the plate washed 3 x with TBS containing 0.1% (v/v) Tween-20 (wash buffer) and blotted on paper towels. The amount of bound anti-My9-6 antibody was detected with either goat anti-mouse IgG-HRP or donkey anti-human IgG-HRP diluted 1:1000 with blocking buffer in the appropriate wells. These secondary antibodies were incubated on the assay plate for one hour at room temperature, protected from light. The plate was washed and blotted as before. The plate was developed using TMB (BioFX Laboratories, Randallstown, MD, cat. no. TMBW-0100-01), then quenched with Stop solution (BioFX Laboratories, Randallstown, MD, cat. no. STPR-0100-01). The assay plate was read at A450nm using a

| TITERTEK[®] Titertek Multiskan Plus MK II plate reader (Huntsville, AL).

Please replace paragraph no. 196 with the following amended paragraph:

HL-60 membranes, prepared as described above, were dried down from a starting concentration of 10 µg/mL in deionized water on the polystyrene surface of Immulon 2 96-well assay plates (Dynex Laboratories, Chantilly, VA), using a Labconco vacuum desiccator (Labconco, Corp, Kansas City, MO), overnight at room temperature. The ELISA plate was then blocked with a 1% fraction V bovine serum albumin (Sigma-Aldrich, Inc., St. Louis, MO, cat. no. A-3294), 0.05% Tween-20 (Sigma-Aldrich, Inc., St. Louis, MO, cat. no. P-2287) solution in Tris-buffered saline (50 mM tris, 150 mM sodium chloride, pH 7.5, TBS), 300 µl per well, at 37°C for 1 hour. Following this blocking step, the plate was drained of blocking buffer and blotted onto paper towels. Two twofold serial dilutions of test or control reagents were prepared in blocking buffer, in quadruplicate, starting at 1.25×10^{-8} M titrated down to 2.44×10^{-11} M (2x the final concentration needed) on a flexible 96-well assay plate. These unlabeled competing reagents were then mixed with an equal volume of 2.5×10^{-10} M biotinylated murine anti-My9-6 (ImmunoGen, Inc., Cambridge, MA); the positive control contained no competing reagent, whereas the negative control contained blocking buffer alone. 50 µL of these mixtures was transferred to designated wells on the membrane-coated assay plate that was then incubated overnight at 4°C. The well contents were then aspirated into a waste flask containing 10% (v/v)

bleach, and the plate washed 3 x with TBS containing 0.1% (v/v) Tween-20 (wash buffer). The plate was blotted onto paper towels, and the amount of bound biotinylated murine anti-My9-6 was detected with 100 µL per well of streptavidin-alkaline phosphatase (Jackson ImmunoResearch Laboratories, West Grove, PA, cat. no. 016-050-084), diluted 1:5,000 in blocking buffer. Following a one hour incubation at room temperature and protected from light, the unbound secondary antibody reagent was washed from the wells, and the plate was developed using TMB (BioFX Laboratories, Randallstown, MD, cat. no. TMBW-0100-01) quenched with Stop solution (BioFX Laboratories, Randallstown, MD, cat. no. STPR-0100-01).

The assay plate was read at A450nm using a TITERTEK Titertek® Multiskan Plus MK II plate reader (Huntsville, AL).

Please replace paragraph no. 201 with the following amended paragraph:

The cumulative results from the various cDNA clones and the peptide sequence analysis provided the final murine My9-6 light and heavy chain sequences presented in Figure 8. Using Kabat and AbM definitions, the three light chain and heavy chain CDRs were identified (Figures 8 and 9). A search of the NCBI IgBlast database indicates that the muMy9-6 antibody light chain variable region most likely derives from the mouse IgV₂κ 8-27 germline gene while the heavy chain variable region most likely derives from the IgV_H V102 germline gene (Figure 10).